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Original paper

**Social correlates of variation in urinary oxytocin concentrations of mantled howler monkeys *Alouatta palliata* (Gray, 1849) (Primates: Atelidae): A preliminary assessment**

**Correlatos sociales de la variación en las concentraciones de oxitocina urinaria de monos aulladores de manto *Alouatta palliata* (Gray, 1849) (Primates: Atelidae): Una evaluación preliminar**

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**ABSTRACT.** There is growing evidence that non-human primate sociality is linked to proximate neuroendocrine mechanisms. Arginine vasopressin, endorphins, and oxytocin may be involved in such mechanisms. Here, we perform a preliminary analysis of the social correlates of variation in urinary oxytocin concentrations in mantled howler monkeys (*Alouatta palliata*). From January to December 2017, we studied 13 adult mantled howler monkeys belonging to two groups. We recorded the occurrence of social interactions (547.5 observation hours); assessed proximity among group members (2,194 instantaneous recordings); and collected 172 urine samples. Urine samples were assayed (ELISA) for oxytocin concentrations, which were corrected for specific gravity, resulting in 54 analyzed samples. Hormone concentrations increased by approximately 62% when individuals were involved in affiliative interactions. With respect to when no affiliative interactions occurred, urinary oxytocin concentrations were higher when



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dyads with a low-quality social relationship affiliated. These results agree with previous contentions that this hormone is linked to participation in affiliative interactions. Convergence between these and results from other taxa supports the hypothesis that the biological mechanisms that allow for primate sociality are shared among species.

**Key words:** affiliation; *Alouatta*; neuroendocrine mechanisms; platyrrhines; sociality

**RESUMEN.** Existe una creciente evidencia de que la sociabilidad en primates no humanos está relacionada con mecanismos neuroendocrinos. La arginina vasopresina, las endorfinas y la oxitocina pueden estar involucradas en tales mecanismos. Aquí, realizamos un análisis preliminar de los correlatos sociales de la variación en las concentraciones de oxitocina urinaria en monos aulladores de manto (*Alouatta palliata*). De enero a diciembre de 2017, estudiamos 13 monos aulladores de manto adultos pertenecientes a dos grupos. Registramos la ocurrencia de interacciones sociales (547.5 horas de observación); evaluamos la proximidad entre los miembros del grupo (2.194 registros instantáneos); y recolectamos 172 muestras de orina. Analizamos las muestras de orina (ELISA) para determinar las concentraciones de oxitocina, las cuales corregimos por la gravedad específica, lo que resultó en 54 muestras analizadas. Con respecto a cuando no ocurrieron interacciones afiliativas, las concentraciones de oxitocina aumentaron aproximadamente un 62% cuando los individuos participaron en interacciones afiliativas, y las concentraciones de oxitocina fueron mayores cuando diádas con una relación social de baja calidad se afiliaron. Estos resultados convergen con propuestas previas de que la oxitocina está vinculada a la participación en interacciones afiliativas. La convergencia entre estos resultados y los reportados para otros taxa apoya la hipótesis de que los mecanismos biológicos que permiten la sociabilidad de los primates se comparten entre las especies.

**Palabras clave:** afiliación; *Alouatta*; mecanismos neuroendocrinos; platirrininos; sociabilidad

## INTRODUCTION

Most diurnal primates live in permanent social groups, where individuals enjoy benefits but also face costs (Alberts, 2018). Given that competition is a pervasive consequence of group living and that it may negatively affect survival and reproduction (e.g., Altmann & Alberts, 2003; Packer *et al.*, 1995), mechanisms that favor the maintenance of sociality despite competition should be under strong selective pressure (Aureli *et al.*, 2012). The proximate neuroendocrine mechanisms underlying sociality in non-human primates have been explored (Massen *et al.*, 2010), and there is indirect evidence that arginine vasopressin, endorphins, and oxytocin may be involved in such mechanisms (Insel, 2010).

In mammals, oxytocin is linked to female reproductive physiology, particularly in the peripartum period: it is responsible for uterine contractions during labor; it stimulates milk ejection; and it is involved in mother-offspring bonding and maternal behavior (Anestis, 2010). However, oxytocin also plays an important role in the formation of social bonds and social preferences, social recognition, and trust (Beery & Zucker, 2010; Meyer-Lindenberg *et al.*, 2011; Anacker & Beery, 2013; Benarroch, 2013; Crockford *et al.*, 2013). This hormone may favor sociality through its

influence on the brain reward system: oxytocin release results in increased dopamine concentrations, which are involved in reward and pleasure processes (Love, 2014). These processes in turn affect social bonds with conspecifics because dopamine influences the assignment of motivational value or relevance (Liu & Wang, 2003; Love, 2014). Thus, oxytocin may be part of a positive feedback mechanism underlying socialization. This mechanism is both associated with positive interactions with peers (i.e., affiliation) and agonistic behavior (De Dreu, 2012; Anacker & Beery, 2013; Fujii *et al.*, 2016; Samuni *et al.*, 2016; Benítez *et al.*, 2018; Cavanaugh *et al.*, 2018; Rincon *et al.*, 2020). In the latter, oxytocin facilitates the categorization of individuals as belonging to the same or another group, promoting cooperation and trust toward ingroup peers, but fear and distrust towards outgroup individuals (De Dreu, 2012; Fujii *et al.*, 2016).

Here, we perform a preliminary analysis of the social correlates of variation in urinary oxytocin concentrations in mantled howler monkeys (*Alouatta palliata*). Howler monkeys live in groups with relatively stable composition and high spatial cohesion (Crockett & Eisenberg, 1987), despite having a low rate of social interactions (Wang & Milton, 2003; Bezanson *et al.*, 2008). Given that the relationship between oxytocin and social behavior has been typically assessed in primate species with comparatively higher rates of social interactions (e.g., cotton-top tamarins, *Saguinus oedipus*: Snowdon *et al.*, 2010; *Pan troglodytes*: Crockford *et al.*, 2013; Witting *et al.*, 2014; capuchin monkeys, *Sapajus apella*: Benítez *et al.*, 2018; *Barbary macaques*, *Macaca sylvanus*: Rincon *et al.*, 2020), howler monkeys are an interesting model for examining convergence and divergence in the proximate mechanisms underlying primate sociality (Anacker & Beery, 2013). We hypothesized that variation in urinary oxytocin concentrations of mantled howler monkeys should be linked to social factors. We tested two predictions of this hypothesis. First, as oxytocin is linked to social affiliation and contact, we predicted higher concentrations when individuals exchanged affiliative interactions with peers than when they did not. Second, because oxytocin associates with the expression of social preferences, we predicted higher concentrations following affiliation between peers with a high-quality than between those with a low-quality social relationship.

## MATERIALS AND METHODS

**Study site and subjects.** From January to December 2017, we studied mantled howler monkeys at La Flor de Catemaco, Veracruz, México (18° 26' 39" N, 95° 02' 57" W). The study site is a 250-ha ranch that includes approximately 100 ha of mainly tall evergreen tropical forest. The climate is warm and humid with mean annual temperature of 25°C and rainfall of 2,600 mm. We studied 13 adult mantled howler monkeys belonging to two groups (group 1 = three adult males and three adult females, one juvenile, and one infant; group 2 = three adult males and four adult females, two juveniles, and one infant). Studies at this site began in 2004 and since 2012 groups are sampled on a weekly basis. Subjects are fully habituated to the presence of researchers (i.e., they pay no attention to us, and our presence does not interfere with their behavior) and are easily recognized through anatomical and physiognomic characteristics.

**Behavioral sampling.** Behavioral sampling was conducted by a single observer with support from two to three field assistants. We recorded all-occurrences (Altmann, 1974) of affiliative (i.e., friendly and peaceful interactions exchanged between individuals with the function of developing,

maintaining, or strengthening social bonds: Jasson del Toro & Nekaris, 2019) and agonistic (i.e., social interactions that involve threatening, aggression, fighting, or submission: Young, 2019) social interactions as described in the ethogram of Dias & Rangel-Negrín (2015; Table 1). We noted the type of interaction that was exchanged, the identities of the subjects that were involved, and each subject's role as either actor or receptor. We used scan sampling with instantaneous recording (Altmann, 1974) repeated at 15 min intervals to study proximity among all adult group members according to the following categories: contact; <1 m; 1–5 m; >5 m (Van Belle *et al.*, 2008; Wang & Milton, 2003). We completed a total of 547.5 focal observation hours (group 1 = 288.5 h; group 2 = 259 h) and of 2,194 instantaneous recordings (group 1 = 1,174; group 2 = 1,020).

**Table 1.** Social interactions recorded in this study.

Type of interaction	Behavior	Description	Rate (int./h)
Affiliative	Contact	Actor places some part of its body in contact with the receptor's body	0.117
	Grooming	Actor touches some part of the receptor's body with its fingers and gently manipulates its hair	0.005
	Hugging	Actor places its arms around the receptor's body	0.005
	Playing	Individuals interact with rapid movements and physical contact, including touching, biting, and chasing without harming each other	0.152
	Playing invitation	Actor approaches the receptor, reaching for him with its hands and/or feet or with other quick movements	0.002
	Scratching	Actor repeatedly moves its hand in some part of the receptor's body	0.002
	Touching	Transient contact (<5 s) between some part of the actor and the receptor's body	0.007
Agonistic	Chasing	Actor moves toward a fleeing receptor	0.018
	Slapping	Actor hits the receptor with an open hand	0.004
	Supplanting	Actor approaches the receptor and occupies its position after the latter leaves	0.022
	Threatening	Actor shakes and/or breaks branches looking toward the receptor. May include vocalizations (roaring and barking) and charges	0.031

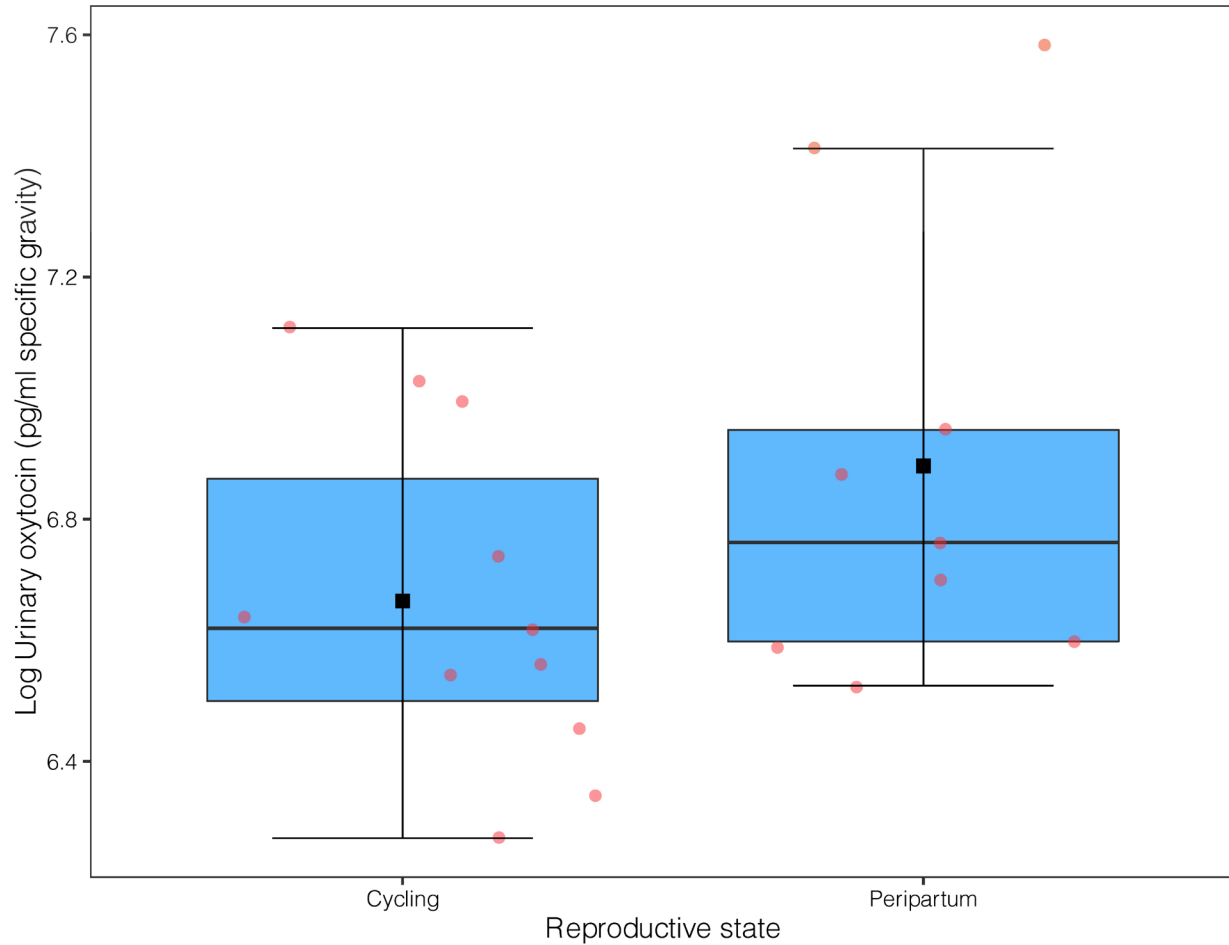
**Urine collection and analysis.** We determined oxytocin concentrations in urine samples. We collected 172 samples immediately after deposition that were matched with individuals and were uncontaminated by soil or feces as well as by urine from other individuals. We collected urine with insulin syringes from a plastic sheet positioned underneath subjects during urination or directly from vegetation to 1.5 ml Eppendorf microcentrifuge tubes. We labeled each tube with subject identity, date, and hour of collection and placed tubes in a cooler with gel ice packs while in the

field. We stored samples in a freezer at  $-20^{\circ}\text{C}$  at the field station (Catemaco, Veracruz) and shipped them in dry ice at the end of fieldwork to the Primate Behavioral Ecology Lab at the Instituto de Neuroetología (Xalapa, Veracruz), where they were stored again at  $-40^{\circ}\text{C}$ . Time of collection (linear mixed model of hour of collection on oxytocin concentrations per sample with subject identity as a random factor:  $\chi^2 = 0.13$ ,  $P = 0.723$ ) and storage duration (linear mixed model of days to assay on oxytocin concentrations per sample with subject identity as a random factor:  $\chi^2 = 0.01$ ,  $P = 0.961$ ) did not affect oxytocin concentrations.

We determined oxytocin concentrations in unextracted urine samples with an enzyme-linked immunosorbent assay (ELISA) at the Instituto de Investigaciones Cerebrales (Universidad Veracruzana). We used a commercial kit (Oxytocin ELISA Kit (ab133050), Abcam PLC, UK; sensitivity = 15 pg/ml, calibration range = 15.6-1,000 pg/ml) in an IMARK microplate reader (Bio-Rad Laboratories, Inc., CA, USA) following the manufacturer's instructions. The antibody in this kit is highly specific for oxytocin, showing low cross-reactivity with [Arg8]-vasotocin (7.5%) and mesotocin (7%). Although this assay has not been tested before in howler monkeys, it has been validated in various primate species, including humans, tamarin monkeys, and chimpanzees (Bick & Dozier, 2010; Snowdon *et al.*, 2010; Witting *et al.*, 2014). We ran all samples in duplicate. Serial dilutions of five samples yielded results that ran parallel to the kit's standards ( $R^2 = 0.8$ ,  $F = 1.4$ ,  $P = 0.265$ ). Mean recovery of oxytocin was 89.7% ( $N = 6$ ). Mean coefficient of variation for intraassay replicates was 7.1% ( $N = 6$ ) and 7.5% for inter-assay replicates ( $N = 3$ ).

We conducted a biological validation of collection, preservation, and assay methods by comparing oxytocin concentrations between females in the peripartum period and cycling females. Specifically, we predicted that peripartum females should have higher oxytocin concentrations than cycling females due to the involvement of oxytocin in labor and lactation (Kumaresan *et al.*, 1979; Spinolo *et al.*, 1992; Neuman, 2003). We classified females in peripartum from one week before to one week after parturition. For this validation, we used samples that were not associated with participation in social interactions and that were not used in further analysis ( $N$  cycling = 11,  $N$  peripartum = 9). Females in the peripartum period had urinary oxytocin concentrations that were on average 25% higher than those of cycling females (linear mixed model with female identity as a random factor:  $\chi^2 = 2.13$ ,  $P = 0.041$ , 95% C.I. = 0.01–1.01; Fig. 1). Thus, our procedures allow assessing variation in urinary oxytocin of mantled howler monkeys associated with the biological functions of this hormone.

We corrected oxytocin concentrations by specific gravity to account for variation among samples in urine volume and concentration (Miller *et al.*, 2004). Each sample's specific gravity was divided by mean specific gravity across all samples (1.02) and this value was multiplied by oxytocin concentration. We determined specific gravity with a pocket refractometer (PAL-10S, Atago, U.S.A., Inc., WA, USA). One hundred and eighteen samples did not have enough volume to be analyzed for both oxytocin and specific gravity, so we report results for 54 samples (mean  $\pm$  SD =  $4.2 \pm 1.9$  samples per subject).



**Figure 1.** Variation in urinary oxytocin concentrations (corrected for specific gravity) between cycling and peripartum mantled howler monkey females (*Alouatta palliata*). Thick lines inside the boxes are the medians; black squares are the means; box limits are the 25<sup>th</sup> and 75<sup>th</sup> percentiles; whiskers indicate 1.5\*interquartile ranges; data points are red circles.

**Data organization.** Following previous studies that have demonstrated a 15–60 min latency from the exogenous administration of oxytocin to an elevation in urinary levels of this hormone (humans: Amico *et al.*, 1987; marmosets: Seltzer & Ziegler, 2007; capuchin monkeys: Benítez *et al.*, 2018), we classified each sample as associated (15<collection<60 min from an interaction) or not associated (15>collection>60 min from an interaction) with the occurrence of affiliative interactions.

We defined a composite index of social relationship quality for each dyad with the following measures: 1) rate of affiliative interactions, calculated as the frequency of affiliation divided by the observation time for each dyad; 2) rate of agonistic interactions, calculated as the frequency of agonism divided by the observation time for each dyad; 3) reciprocity in the exchanging of affiliative interactions as:

$$\text{Reciprocity} = \frac{\text{Affiliation A} - \text{Affiliation B}}{\text{Affiliation A} + \text{Affiliation B}}$$

Where *Affiliation A* is the number of times individual *A* directed affiliative interactions at *B*, and *Affiliation B* is the number of times individual *B* directed affiliative interactions at *A*. This index ranges between 1 (*A* was always the actor) and -1 (*B* was always the actor), with zero indicating absolute reciprocation (Ryu *et al.*, 2014); 4) proximity as:

$$\text{Dyadic proximity index} = \frac{\sum_1^n (\text{contact} * 1) + (< 1\text{m} * 0.67) + (1-5\text{m} * 0.33) + (> 5\text{m} * 0)}{\text{Total number of instantaneous recordings for the dyad}}$$

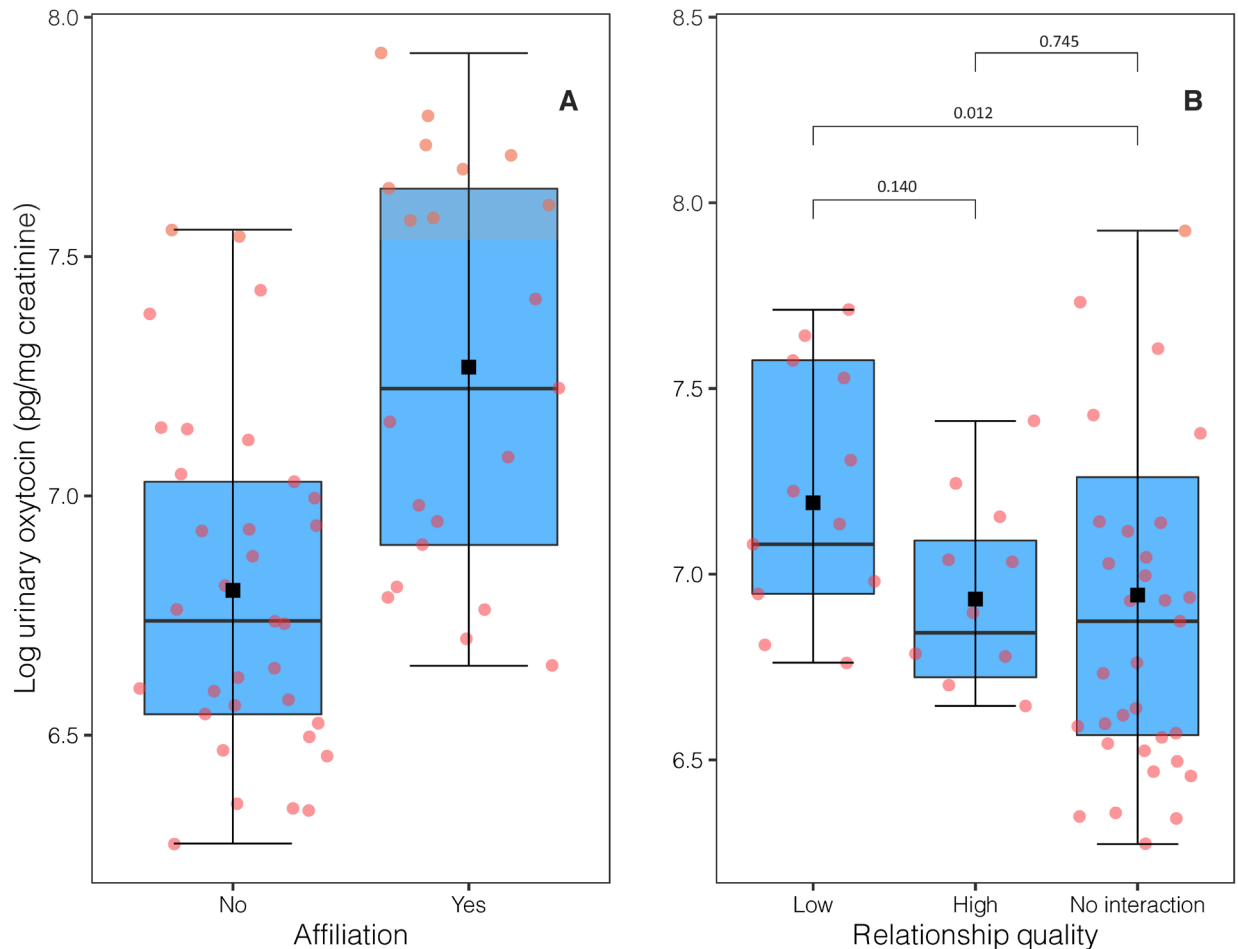
In this index, we summed the frequency of recordings in which the dyad was observed in each proximity category after multiplying frequencies by a decreasing weighing factor, such that a dyad always observed in contact would have an index of 1 whereas one that was always >5 m would have an index of 0 (González-Hernández *et al.*, 2014). We then divided this sum by the total number of instantaneous recordings for the dyad. These four measures proxy three components of social relationship quality: value (affiliation), compatibility (agonism and proximity), and security (reciprocity; Cords & Aureli, 2000). We determined the quartiles of the four measures and assigned a value of 1 to each of the following quartiles: 1) third quartile of affiliation rate; 2) third quartile of proximity index; 3) first quartile of agonism rate; 4) first quartile of reciprocity index. Other quartiles were assigned a value of 0. Our composite measure of social relationship quality was the sum of quartile values, and we considered that dyads with a quartile sum >2 had a high-quality social relationship.

**Data organization and analysis.** We used a linear mixed model to assess variation in log-transformed urinary oxytocin concentrations as a function of the participation in affiliative interactions (categorical predictor with two levels: yes, no) and social relationship quality of dyads that participated in affiliative interactions (categorical predictor with three levels: high-quality, low-quality, sample not associated with affiliative interaction). In this model, as well in the biological validation and temporal variation in hormone concentrations mentioned above: 1) we added identity as a random factor to account for repeated sampling of subjects; 2) assessed model fit visually via Q-Q plots and Shapiro-Wilks tests of model residuals, which indicated residual normality in all cases; 3) and to determine whether the fixed factors explained more variation in hormone concentrations than the random factors alone, we compared the complete models (i.e., with fixed and random factors) with a null model including only the response and the random variable with a likelihood ratio test (Pinheiro & Bates, 2000). These tests were significant in all cases ( $P < 0.001$ ), indicating a limited influence of the random factors on model results. Given the small number of samples that were analyzed, we diagnosed model stability via DFBETAS which indicated that model results were not affected by influential cases. We ran *post-hoc* Tukey pairwise comparisons to assess the significant influence of social relationship quality on urinary oxytocin concentrations. All analyses were performed with R 4.0.0. (R Core Team, 2020).



## RESULTS

Urinary oxytocin concentrations varied between 530.2 and 2,765.2 pg/ml with a mean of  $1,192.4 \pm 567.4$  pg/ml. The mixed model explained 50% ( $R^2 = 0.50$ ) of the deviance in urinary oxytocin concentrations. Variation in urinary oxytocin concentrations was significantly related to the exchanging of affiliative interactions ( $\chi^2_1 = 33.0$ ,  $P < 0.001$ , 95% CI = 0.54–1.16; Fig. 2A) and the quality of the social relationship of dyads ( $\chi^2_2 = 13.1$ ,  $P = 0.001$ , 95% CI = 0.29–1.09; Fig. 2B). Specifically, urinary oxytocin concentrations increased by approximately 62% when individuals were involved in affiliative interactions and, with respect to when no affiliative interactions occurred, urinary oxytocin concentrations were higher when dyads that had a low-quality relationship affiliated.



**Figure 2.** The influence of participation in affiliative interactions (A) and dyadic social relationship quality (B) on urinary oxytocin concentrations (corrected for specific gravity, SP) in mantled howler monkeys (*Alouatta palliata*). Thick lines inside the boxes are the medians; black squares are the means; box limits are the 25th and 75<sup>th</sup> percentiles; whiskers indicate 1.5\*interquartile ranges; data points are red circles. In B, *post-hoc* pairwise comparisons were performed with Tukey contrasts.

## DISCUSSION

This is the first study to assess the relationship between social factors and oxytocin concentrations in howler monkeys. We found that hormone concentrations were higher when individuals affiliated than when no affiliation occurred and among dyads with low-quality social relationships that exchanged affiliation than when no affiliation occurred. Given the limited set of urine samples that could be assayed, these results provide preliminary evidence of the involvement of oxytocin in the regulation of the social behavior of mantled howler monkeys.

Urinary oxytocin concentrations of mantled howler monkeys were higher when individuals participated in affiliative interactions, a pattern also observed in capuchin monkeys, chimpanzees, common marmosets, Barbary macaques, and humans (Seltzer & Ziegler, 2007; Morhenn *et al.*, 2008; Witting *et al.*, 2014; Benítez *et al.*, 2018; Rincon *et al.*, 2020). However, whereas the relationship between oxytocin concentrations following affiliation and the quality of social relationships is positive in chimpanzees (Crockford *et al.*, 2013), it was negative in mantled howler monkeys. It must be noted, however, that howler monkeys affiliate at low rates compared to other primates and, contrary to chimpanzees, rarely groom (Di Fiore & Campbell, 2007). Furthermore, most samples analyzed here that were associated with low-quality social relationships corresponded to affiliative interactions between males. In several howler monkey species, female dyads and female-male dyads have the strongest social bonds, whereas male-male interactions are infrequent and mostly agonistic (Di Fiore & Campbell, 2007). It is thus possible that oxytocin is an influential mechanism in sociality among male howler monkeys. In this context, increased oxytocin concentrations may facilitate social interactions between males via anxiolytic effects, as proposed for Barbary macaques (Rincon *et al.*, 2020). Given the small number of samples that were assayed here, examination of this possibility remains for future research.

Measurement of peripheral oxytocin in urine samples of non-human primates commonly involves a purification step (e.g., Benítez *et al.*, 2018; Crockford *et al.*, 2016). Extraction of urine samples reduces interference from other compounds, usually resulting in lower oxytocin concentrations in extracted than in unextracted samples (Ziegler, 2018). We did not purify urine of mantled howler monkeys, so our results pertain to oxytocin concentrations in unextracted samples. Thus, it must be determined if the association between oxytocin concentrations and social behavior reported here is also found when assaying extracted urine samples. Nonetheless, (i) significant oxytocin response in the 15-60 min period following affiliation that converges with studies based on extracted and unextracted urine samples (e.g., Boose *et al.*, 2018; Crockford *et al.*, 2013), (ii) positive results for the biological validation, (iii) positive results for analytical validations, and (iv) the specificity of the antibody that we used, seem to support the reliability of our methods.

## CONCLUSIONS

We provide preliminary evidence that variation in urinary oxytocin concentrations of mantled howler monkeys is associated with social factors. Specifically, oxytocin increases after the participation of individuals in affiliative interactions and is linked to the quality of social relationships between individuals that affiliate. Similarity between these and results from other

taxa support the hypothesis that the biological mechanisms underlying primate sociality are shared among species.

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